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Plasminogen activator inhibitor activity and other fibrinolytic variables in patients with coronary artery disease

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SUMMARY Several fibrinolytic variables, including plasminogen activator inhibitor activity, were studied before and after exercise in 67 normolipidaemic patients with coronary artery disease and in 25 hyperlipidaemic patients with coronary artery disease. Before exercise plasminogen activator inhibitor activity was higher in the patient groups than in a group of 10 healthy volunteers. For those who were normolipidaemic plasminogen activator inhibitor activity was greater in patients with angina pectoris who had had a myocardial infarction. The concentration of antigenic tissue-type plasminogen activator was similar in all the patients with coronary artery disease and higher than in the control group. After the exercise test fibrinolytic capacity was lower in the patients with angina pectoris and a previous history of myocardial infarction. After exercise both the released immunological tissue-type plasminogen activator and fibrinolytic capacity were lower in the hyperlipidaemic patients than in the normolipidaemic patients. The concentration of plasminogen activator inhibitor was also higher in the hyperlipidaemic patients. Patients with hyperlipidaemia IV had the highest plasminogen activator inhibitor activity. The increase in plasminogen activator inhibitor activity found in the patients was partially inhibited by antiserum against plasminogen activator inhibitor-1 in vitro. The formation of a complex of about 115 000 daltons between plasminogen activator inhibitor and purified tissue-type plasminogen activator was detected by a zymographic fibrin technique.

These findings show that in patients with coronary artery disease fibrinolytic activity is impaired by an increase in plasminogen activator inhibitor. Impaired fibrinolysis may be related to the clinical evolution of coronary artery disease in these patients.

Fibrinolytic activity is impaired in many patients with coronary artery disease.[1-3] This impairment is detectable both in the resting state[4-6] and after physical exercise or venous occlusion.[7-9] It has been suggested that impaired fibrinolysis has a pathogenic role in coronary artery disease.[10] Impaired fibrinolysis could be caused by an increase in the activity of plasminogen activator inhibitor[11 12]; however, some groups have reported only a very occasional and transient increase in plasminogen activator inhibitor in patients with coronary artery disease.[13] In 1985 several groups reported increased concentrations of plasminogen activator inhibitor in patients with coronary artery disease and suggested that this increase may have a role in the pathogenesis of this disorder.[14-16]

The pathogenic role of plasminogen activator inhibitor in coronary artery disease was confirmed recently when a higher rate of reinfarction was found in patients with coronary artery disease with high concentrations of plasminogen activator inhibitor.[17 18] Others, however, reported no correlation between concentrations of plasminogen activator inhibitor and the severity of coronary disease.[15]

It has also been reported that hyperlipidaemia is usually associated with a decrease in fibrinolytic

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activity,[19 20] especially when it is caused by an increase in low density lipoproteins. Increased concentrations of high density lipoproteins were reported to have the opposite effect, that is they activated fibrinolysis.[21] These results were not, however, confirmed in another study.[22] It is clear, however, that patients with coronary artery disease who also have hyperlipidaemia generally have increased concentrations of plasminogen activator inhibitor, especially if the hyperlipidaemia is characterised by an increase in triglycerides,[15] whereas plasminogen activator inhibitor concentrations are usually normal in cases of hypercholesterolaemia.[16 23] Some workers found no correlation between triglyceride concentrations and plasminogen activator inhibitor in normal subjects.[24]

These data prompted an examination of possible correlations between the various clinical types of coronary artery disease and the concentration of plasminogen activator inhibitor and other fibrinolytic variables. We paid special attention to evaluating to what extent the presence or absence of a previous history of myocardial infarction was related to concentrations of plasminogen activator inhibitor in patients with angina.

Patients and methods

STUDY GROUPS

There were 67 men in the normolipidaemic group with coronary artery disease (group 1). None had hyperlipidaemia, hyperglycaemia, or severe hypertension. These patients were classified in three groups: (*a*) patients with angina pectoris without a previous history of myocardial infarction (n = 15); (*b*) patients with angina and a previous history of myocardial infarction (n = 14), and (*c*) patients who had had a myocardial infarction about three weeks before this study (n = 38).

Myocardial infarction or angina were diagnosed according to clinical, electrocardiographic, and enzymatic criteria.

We also studied 25 hyperlipidaemic men with coronary heart disease (group 2). These patients were classified into three groups as having hyperlipidaemia IIa, IIb, or IV. There were 10 healthy men in the control group. None had a history of angina or electrocardiographic evidence of ischaemic heart disease. Biochemical variables, particularly blood sugar and cholesterol concentrations, were normal.

All the subjects underwent a symptom limited bicycle ergometer test in a sitting position, starting with a workload of 30 W for three minutes. The workload was gradually increased by 25 W every three minutes. Each subject attained 85% of the individually expected maximal working capacity.

Table 1 shows characteristics of all the groups.

REAGENTS

The reagents for measuring functional protein C were obtained as described elsewhere.[25] Other reagents used were: chromogenic substrate S-2251 and human fibrinogen (Kabi Diagnostica, Stockholm), and bovine thrombin (Behringwerke AG, Marburg, West Germany). Human plasminogen was purified by lysine-sepharose chromatography,[26] followed by gel filtration on Sephadex G-150. Purified human single-chain tissue plasminogen activator from human melanoma cell culture (Biopool, Sweden) was used to measure the activity of plasminogen activator inhibitor. Human tissue-type plasminogen activator was also purified in our laboratory from Bowes-melanoma cell-culture conditioned medium, as described elsewhere.[27 28] It was purified without the addition of aprotinin and was obtained as double (75%) and single chain molecules (25%) and had a specific activity of 250 000 IU/mg

Table 1 *Characteristics of the study groups (mean (SD))*

	No	*Age*		*No of subjects with mild hypertension**	*No of subjects who were habitual smokers†*	*No of subjects of sedentary habits*	*Submaximal workload (W)*
		Mean	*Range*				
Group 1 (normolipidaemic):	67	54·6 (7·9)	(36–69)	22	39	17	115·6 (21·6)
Group A	15	55·9 (7·6)	(36–67)	6	6	3	109·4 (22·5)
Group B	14	56·7 (7·7)	(44–66)	4	3	2	119·0 (19·7)
Group C	38	52·5 (5·8)	(37–67)	12	30	12	116·8 (22·1)
Group 2 (hyperlipidaemic):	25	53·5 (8·8)	(37–69)	3	20	7	112·0 (23·0)
IIa	11	52·3 (8·2)	(42–69)	1	10	2	113·1 (29·7)
IIb	6	53·2 (13·5)	(37–68)	0	6	1	97·2 (11·4)
IV	8	56·2 (5·9)	(53–67)	2	4	4	124·9 (9·1)
Control group	10	49·3 (4·9)	(43–59)	0	8	3	153·3 (20·5)

*Mild hypertension: systolic blood pressure between 140–160 mm Hg and diastolic blood pressure up to 100 mm Hg.
†More than three cigarettes/day.

when measured by a clot lysis assay[28] with an international tissue-type plasminogen activator standard (National Institute for Biological Standards and Control reagent 83/517). Rabbit anti-bovine endothelial cell plasminogen activator inhibitor-1[29 30] and rabbit anti-plasminogen activator inhibitor-2 sera (Batch R3, approximate titre 150 μg/ml) were kindly donated by Dr D J Loskutoff (California, USA) and Dr E K O Kruithof (Lausanne, Switzerland) respectively. All other chemicals were of analytical grade.

METHODS

Venous blood samples at rest were obtained between 8.30 am and 9.30 am. Subjects remained in a sitting position for 10 to 15 minutes before venepuncture. Blood samples were obtained immediately after the exercise test. Blood was obtained and anticoagulated for the different tests as previously described.[31]

Whole serum cholesterol and triglycerides were measured by enzymatic methods. Protein C antigen was measured by an enzyme-linked immunosorbent assay method (Diagnostica Stago, France). The amidolytic and coagulant activities of protein C were assayed as previously described.[25] Antithrombin III was measured by an amidolytic method.[32]

Plasma euglobulin lysis time was measured by Buckell's method.[33] Fibrinolytic euglobulin activity was also measured on plasminogen-rich fibrin-agar plates as previously described.[14] Plasminogen and fast-acting antiplasmin activities were measured by chromogenic substrate methods.[34 35] Concentrations of fibrinogen/fibrin degradation products were measured by a haemagglutination inhibition method.[36] The activity of tissue-type plasminogen activator was measured by a functional method.[37] Plasminogen activator inhibitor activity was assayed as previously described.[37 38] One unit of inhibitor was defined as the amount that inhibits 1 IU of tissue-type plasminogen activator in 10 minutes (tissue-type plasminogen activator was calibrated against the international tissue-type plasminogen activator standard). Tissue-type plasminogen activator antigen was measured by an enzyme-linked immunoadsorbent assay method[39] (Biopool, Sweden), after acidification and neutralisation of plasma as previously described.[39]

The formation of tissue-type plasminogen activator/inhibitor complexes was studied by a zymographic fibrin overlayer technique[40] with a 7·5% polyacrylamide separating gel and a 4% polyacrylamide stacking gel. A volume (25 μl) of several dilutions of plasma was mixed with 2.5 μl of tissue-type plasminogen activator (25 U/ml). In another experiment 25 μl of a fixed dilution of plasma was mixed with several concentrations of tissue-type plasminogen activator. The mixtures were incubated for 10 minutes at 37°C. Then 25 μl of a buffer containing 0·125 mol/l TRIS-HCl, 4% sodium dodecyl sulphate, and 20% glycerol (pH 6·8) was added and the mixture was incubated for 15 minutes at 37°C. The samples were laid on the stacking gel and run at 25 mA for 3–4 hours at 4°C. The gels were washed for one hour in 2·5% Triton X-100 and for 20 minutes in deionised water. Fibrinolytically active bands were revealed by placing the gels on a plasminogen-rich fibrin-agarose gel and incubating them at 37°C in a humid environment for 18 hours. The molecular weight standards used were bovine serum albumin (68 000), phosphorylase (93 000), and β galactosidase (130 000).

The inactivation of plasminogen activator inhibitor activity by anti-plasminogen activator inhibitor-1 and anti-plasminogen activator inhibitor-2 was studied in three plasma samples from patients with ischaemic coronary disease and high plasminogen activator-inhibitory activity. Diluted plasma (50 μl, 1/4) was incubated with 5 μl of either diluted antibody (1/2) or diluted non-immune rabbit serum (1/2) or buffer for 15 minutes at 37°C. The residual inhibitory activity was assayed with tissue-type plasminogen activator (75% two chain tissue plasminogen activator) as described above.[37 38]

Mann-Whitney U and Student's *t* tests were used for statistical analysis.

Results

Concentrations of antithrombin III, protein C (functional and immunological), plasminogen, fibrinogen/fibrin degradation products, and α_2-antiplasmin at rest and after exercise were not significantly different in the clinical patient groups nor were there any differences between the control group and the patients.

Concentrations of immunological tissue-type plasminogen activator (table 2) were similar in groups A, B, and C, that is slightly higher than in the control group. In contrast, the concentration of immunological tissue-type plasminogen activator released after the exercise test was lower in patient groups A and B than in group C. The value in group C was similar to that of the control group. On the other hand, about 50% of the patients in group C had concentrations of released antigenic tissue-type plasminogen activator <3 ng/ml. This was higher than the concentration in the control group.

Fibrinolytic capacity (post-exercise fibrinolytic activity minus fibrinolytic activity at rest) was lower in patients with angina pectoris who had a history of myocardial infarction (group B) than in those with angina pectoris but no myocardial infarction (group

Table 2 *Fibrinolytic variables (mean (SD)) before (B) and after (A) exercise in normolipidaemic patients with coronary artery disease*

	Euglobulin lysis time (min)			*Lysis on fibrin plate (%)*			*Functional t-PA (U/ml)*			*Antigen t-PA (ng/ml)*			*PAI activity (U/ml)*	
	B	*A*	*FC*	*B*	*A*	*FC*	*B*	*A*	*t-PAC*	*B*	*A*	*t-PAR*	*B*	*A*
Group A (n = 15)	248	178	69	113	213	99	0·34	1·31	0·98	12·2	15·5	3·2	2·67†	1·90*
	(60)	(70)	(49)	(97)	(172)	(104)	(0·35)	(1·9)	(1·60)	(3·1)	(5·6)	(4·9)	(2·24)	(2·06)
Group B (n = 14)	220	180	47*	99	157	58	0·29*	0·54*	0·32*	13·0	16·9	3·9	5·20†	4·4†
	(64)	(80)	(55)	(49)	(110)	(71)	(0·29)	(0·52)	(0·35)	(5·6)	(10·4)	(6·4)	(4·97)	(4·3)
Group C (n = 38)	263	154	108	84	211	110	0·27	1·25	0·93	13·2	18·8	5·6	3·7†	2·5*
	(87)	(91)	(91)	(43)	(138)	(147)	(0·43)	(1·70)	(1·41)	(6·3)	(10·0)	(4·0)	(4·2)	(3·4)
Control group (n = 10)	263	143	119	110	185	76	0·48	1·43	0·95	10·1	16·1	5·9	0·69	0·42
	(84)	(59)	(96)	(37)	(116)	(89)	(0·18)	(1·51)	(1·10)	(2·9)	(5·3)	(3·2)	(0·75)	(0·58)

*p < 0·05 compared with control group; †p < 0·02 compared with control group.
Group A, patients with angina pectoris without previous history of myocardial infarction; Group B, patients with angina pectoris and previous history of myocardial infarction; Group C, patients with myocardial infarction about 3 weeks before this study.
FC, fibrinolytic capacity (fibrinolytic activity after exercise minus fibrinolytic activity before exercise); t-PAC, tissue plasminogen activator capacity (T-Pa activity after exercise minus t-PA activity before exercise); t-PAR, t-PA release (antigen t-PA after exercise minus antigen t-PA before exercise); PAI, plasminogen activator inhibitor.

A) or those with recent myocardial infarction (group C) (table 2). This was found for both global assays (euglobulin lysis time and lysis on fibrin plate) and functional tissue-type plasminogen activator concentrations.

Plasminogen activator inhibitor activity at rest was higher in the three patient groups than in the control group. Moreover, 60% of the patients had plasminogen activator inhibitor values above 2 U/ml, a concentration that was never reached by the control subjects.

Patients with angina pectoris and myocardial infarction (group B) had higher plasminogen activator inhibitor activity than the other two groups. Furthermore, in four (28%) of the group B patients concentrations of plasminogen activator inhibitor were > 6 U/ml, a value that none of those in group A reached.

After the exercise test plasminogen activator inhibitor concentrations fell slightly in all the groups examined, but they continued to reflect the values at rest.

Released immunological tissue-type plasminogen activator and fibrinolytic capacity were lower and plasminogen activator inhibitor was higher in hyperlipidaemic patients with coronary artery disease (group 2) than in normolipidaemic patients with coronary artery disease (group 1) (table 3).

In patients with coronary artery disease plasminogen activator inhibitor activity was higher in those with types IIb and IV hyperlipidaemia than in those with type IIa hyperlipidaemia ($p < 0·05$) (table 4). The concentration of plasminogen activator inhibitor was lower in patients with type IIa hyperlipidaemia (approximately 2 U/ml) than in patients with the same pathology but without hyperlipidaemia (approximately 3·7 U/ml).

The residual plasminogen activator inhibitor activity of three plasma samples from coronary artery disease patients with high plasminogen activator inhibitor activity was measured after the addition of anti-plasminogen activator inhibitor-1 and anti-plasminogen activator inhibitor-2. The inhibitory capacity was reduced by anti-plasminogen activator

Table 3 *Fibrinolytic variables (mean (SD)) before (B) and after (A) exercise in various groups of patients with coronary artery disease*

	Euglobulin lysis time (min)			*Lysis on fibrin plate (%)*			*Functional t-PA (U/ml)*			*Antigen t-PA (ng/ml)*			*PAI activity (U/ml)*	
	B	*A*	*FC*	*B*	*A*	*FC*	*B*	*A*	*t-PAC*	*B*	*A*	*t-PAR*	*B*	*A*
Group 1 (n = 61)	255	168	87	92·1	198	99§	0·28	1·11	0·81	12·8	17·6	4·6	3·73§	2·77‡
	(80)	(86)	(80)	(61·6)	(143)	(123)	(0·38)	(1·60)	(1·32)	(5·4)	(9·2)	(5·3)	(4·10)	(3·39)
Group 2 (n = 25)	251	187	64*	106	141	35§	0·24†	0·59*	0·37	12·8	16·8	3·8	5·70§	4·29‡
	(64)	(87)	(74)	(41)	(56)	(45)	(0·23)	(0·87)	(0·75)	(3·0)	(3·9)	(3·2)	(5·40)	(5·03)
Control group (n = 10)	263	143	119	110	185	76	0·48	1·43	0·95	10·1	16·1	5·9	0·69	0·42
	(84)	(59)	(96)	(37)	(116)	(89)	(0·18)	(1·51)	(1·10)	(2·9)	(5·3)	(3·2)	(0·75)	(0·58)

*p < 0·05 compared with control group; †p < 0·012 compared with control group; ‡p < 0·005 compared with control group; §p < 0·05 between group 1 and group 2. Group 1, normolipidaemic patients; Group 2, hyperlipidaemic patients.
See footnote to table 2 for abbreviations.

Table 4 *Fibrinolytic variables (mean (SD)) before (B) and after (A) exercise in hyperlipidaemic patients with coronary artery disease*

	Euglobulin lysis time (min)			*Lysis on fibrin plate (%)*			*Functional t-PA (U/ml)*			*Antigen t-PA (ng/ml)*			*PAI activity (U/ml)*	
	B	*A*	*FC*	*B*	*A*	*FC*	*B*	*A*	*t-PAC*	*B*	*A*	*t-PAR*	*B*	*A*
Group IIa (n = 11)	246 (85·8)	203 (86)	43* (58)	84 (44)	128 (61)	43 (39)	0·16† (0·26)	0·52 (1·16)	0·40 (1·03)	13·1* (3·8)	16·2 (3·8)	3·1 (2·6)	2·0 (4·0)	0·57 (1·37)
Group IIb (n = 6)	260 (36)	174 (90)	87 (95·4)	145 (21)	167 (79)	22 (67)	0·26* (0·19)	0·63 (0·39)	0·37 (0·34)	13·5* (3·1)	17·3 (1·9)	3·8 (1·6)	7·5‡ (4·6)	6·75‡ (4·12)
Group IV (n = 8)	249 (53)	175 (95)	73·7 (76·4)	110 (27)	143 (28)	33 (41)	0·37 (0·14)	0·69 (0·59)	0·32 (0·49)	11·9 (1·4)	17·2 (5·6)	4·8 (5·0)	9·4‡ (4·48)	7·55‡ (5·73)
Controls group (n = 10)	263 (84)	143 (59)	119 (96)	110 (37)	185 (116)	76 (89)	0·48 (0·18)	1·43 (1·51)	0·95 (1·10)	10·1 (2·9)	16·1 (5·3)	5·9 (3·2)	0·69 (0·75)	0·42 (0·58)

*p < 0·05 compared with control group; †p < 0·005 compared with control group; ‡p < 0·001 compared with control group.
See footnote to table 2 for abbreviations.

inhibitor-1 (66% of residual activity) but not by anti-plasminogen activator inhibitor-2 (91% of residual activity). Non-immune rabbit serum did not reduce the inhibitory capacity.

Use of a zymographic technique revealed the formation of a complex between the plasma plasminogen activator inhibitor and purified tissue-type plasminogen activator. The figure shows the formation of a complex between a fixed concentration of tissue-type plasminogen activator and plasma from a patient with coronary artery disease. Formation of the complex was reduced by dilution of the plasma (figure (a)). When we used a fixed dilution of patient plasma we found that as the tissue-type plasminogen activator concentration was increased, the complex also increased (figure (b)). This complex appeared to have a molecular weight of about 115 000 daltons.

Discussion

Our results accorded with those of other workers[14-16] who found that the concentration of basal antigenic tissue-type plasminogen activator at rest is slightly higher in patients with coronary artery disease than in controls. According to Hamsten *et al* this may be the result of a slow but continual release of tissue-type plasminogen activator from the vascular wall,[16] which could reduce the vascular deposits of tissue-type plasminogen activator in these patients and lead to a decreased release of tissue-type plasminogen activator after exercise. Our results seem to confirm this, because release of tissue-type plasminogen activator was lower after exercise in patients with a long history of angina pectoris (groups A and B) than patients with myocardial infarction (group C) in

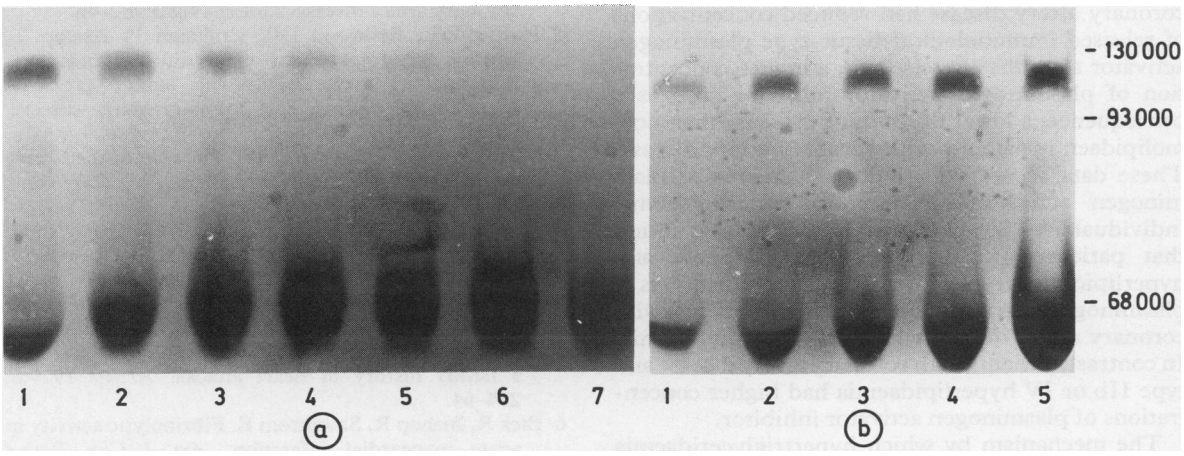

Figure *Fibrin-zymography after sodium dodecyl sulphate polyacrylamide gel electrophoresis of a 10' incubation mixture of tissue-type plasminogen activator with coronary artery disease plasma. (a) Formation of a complex between purified tissue-type plasminogen activator (2·5 µl of 25 U/ml) and 25 µl of several dilutions of plasma (1 = 1/5; 2 = 1/10; 3 = 1/20; 4 = 1/40; 5 = 1/80; 6 = 1/160; 7 = buffer). (b) Formation of a complex between a fixed dilution (1/10) of plasma (25 µl) and 2·5 µl of tissue-type plasminogen activator (1 = 12·5 U/ml; 2 = 18·7 U/ml; 3 = 25 U/ml; 4 = 37·5 U/ml; 5 = 50 U/ml). A complex (115 000 daltons) formed between tissue-type plasminogen activator and the plasma plasminogen activator inhibitor.*

whom tissue-type plasminogen activator release resembled that seen in the control group.

We found that concentrations of plasminogen activator inhibitor activity were higher in patients with coronary artery disease than in controls. This finding accords with the results of earlier studies,[16 19 41] but we also found that patients with angina pectoris and previous myocardial infarction had higher concentrations of plasminogen activator inhibitor than patients with angina and no history of infarction. We believe that the higher concentration of plasminogen activator inhibitor may be related to the development of myocardial infarction in patients with angina pectoris. This suggestion accords with previous reports that reinfarction[17 18] and death[18] are more common in patients with coronary artery disease and high concentrations of plasminogen activator inhibitor.

The increase in plasminogen activator inhibitor may have been responsible for the reduced fibrinolytic capacity that we found in patients with angina pectoris and myocardial infarction (group B). There was no such reduction in patients with angina pectoris with no history of myocardial infarction (group A), even though both groups A and B had similar concentrations of released immunological tissue-type plasminogen activator.

These data support the hypothesis that plasminogen activator inhibitor has a pathogenic effect in patients with coronary artery disease and that this fibrinolytic inhibitor may impair fibrinolysis in these patients.

We found that hyperlipidaemic patients with coronary artery disease had reduced concentrations of released immunological tissue-type plasminogen activator after the exercise test, a higher concentration of plasminogen activator inhibitor and, as a consequence, a lower fibrinolytic capacity than normolipidaemic patients with coronary artery disease. These data accord with reports of increased plasminogen activator inhibitor in hyperlipidaemic individuals.[16] Like Hamsten *et al*[16] we also found that patients with coronary artery disease and hyperlipidaemia type IIa had lower concentrations of plasminogen activator inhibitor than patients with coronary artery disease who were normolipidaemic. In contrast, patients with coronary artery disease and type IIb or IV hyperlipidaemia had higher concentrations of plasminogen activator inhibitor.

The mechanism by which hypertriglyceridaemia could increase plasminogen activator inhibitor is not known[16] but there does seem to be an objective relation between these two variables. We do not know to what extent the possible alteration of the vascular wall in hypercholesterinaemic patients—which is normally more considerable than in hypertriglyceridaemic patients—is related to the decreased release of plasminogen activator inhibitor and tissue-type plasminogen activator.

Nevertheless, even in hypertriglyceridaemic patients with coronary artery disease who had plasminogen activator inhibitor concentrations that were higher than those in normolipidaemic patients with coronary artery disease, released plasminogen activator and the activity of circulating plasminogen activator inhibitor were lower when in addition to being hypertriglyceridaemic the patient was also hypercholesterinaemic (type IIb). The decreased release of tissue-type plasminogen activator that we found in coronary artery disease patients with type IIa hyperlipidaemia does not accord with the findings of Hamsten *et al*[16] who found that tissue-type plasminogen activator release after venous occlusion was adequate. This may be because they studied young patients, who were less likely to have the atherosclerotic alterations than our patients.

In conclusion, our results suggest that the greater increase in plasminogen activator inhibitor in patients with angina pectoris and previous myocardial infarction may be associated with this acute event. It may be that concentrations of plasminogen activator inhibitor in coronary artery disease patients are of prognostic value.

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